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Nodal Signaling: Cryptic Lefty Mechanism of Antagonism Decoded

Dispatch

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The secreted TGFβ factor Lefty antagonizes Nodal signaling during vertebrate embryogenesis, but how it does so has been a mystery. Recent analyses have elucidated the molecular mechanisms underlying this function of Lefty.

The Lefty proteins of the transforming growth factor β (TGFβ) superfamily are essential for mesendoderm development, gastrulation and left–right patterning during vertebrate embryogenesis [1]. Unlike other TGFβs, Lefty does not function via receptor-mediated Smad-dependent signaling, but rather by antagonizing the signaling of at least one other TGFβ subfamily, the Nodals [1]. A balance between Lefty and Nodal activity underlies the proper execution of many developmental processes, as evidenced by the severe and often fatal phenotypes observed in Lefty- or Nodal-deficient embryos [1]. Multiple mechanisms by which Lefty antagonizes Nodal signaling have been suggested, yet direct evidence for such mechanisms has been elusive [2–5]. Similarly, the proposed ability of Lefty to antagonize Nodal-independent signaling remains unconfirmed [3,5–10]. Two recent studies [11,12] have addressed these issues.

Is Lefty a Nodal-Specific Antagonist or an Inhibitor of Multiple TGFβ Signals?

The ability of Lefty to antagonize Nodal signaling has been strongly supported by two key observations. First, the loss of Lefty activity results in an expansion of Nodal signaling in vertebrate embryos [1]. Conversely, the upregulation of Lefty activity leads to a reduction in Nodal signaling [1], as compellingly illustrated by the identical phenotypes of zebrafish that overproduce Lefty and those genetically incapable of Nodal signaling [4,5,13]. Lefty has also been implicated in the antagonism of many other TGFβ signaling pathways — Activin [5,8,9], bone morphogenetic proteins (BMPs) [3,7,10], TGFβ1 [3], Vg1 [7] — as well as the Wnt signaling pathway [6], though the evidence supporting these cases is less substantial than that for Nodal.

Using a Smad2-responsive luciferase reporter assay in cell culture and zebrafish embryos, Chen and Shen [11] and Cheng *et al.* [12] found that Lefty antagonizes Nodal and Vg1 signaling, but not Activin or TGFβ1 signaling. Cheng *et al.* [12] additionally showed that, in zebrafish embryos, Lefty antagonized the ectopic induction of *goosecoid* by Nodal and Vg1 overexpression, but not by Activin. These results suggested that Lefty might specifically antagonize EGF-CFC co-receptor-dependent signaling, as such co-receptors are essential to Nodal/Vg1 signaling [1,14].

The EGF-CFC proteins — Cripto, Cryptic, FRL-1 and Oep — are extracellular glycosylphosphotidylinositol (GPI)-linked factors that interact with Nodal and Vg1/GDF (growth and differentiation factor) ligands and facilitate the ability of these ligands to bind and activate type I (Alk4/7)–type II (ActRIIA/B) transmembrane receptor complexes (Figure 1A) [1,15]. As Lefty only antagonized EGF-CFC-dependent signaling, Cheng *et al.* [12] tested whether Lefty genetically interacts with EGF-CFCs. They found that overexpression of EGF-CFCs antagonized the phenotypic effects of Lefty overexpression.

These correlations between Lefty antagonism and EGF-CGC-dependent signaling led both groups to test whether Lefty biochemically interacts with the EGF-CFC–type I–type II receptor signaling complex. They found that Lefty co-immunoprecipitated with the EGF-CFC co-receptor, but not with the type I or II receptors [11,12]. Furthermore, Lefty competed with Nodal, preventing it from binding EGF-CFCs [11,12]. This competitive binding likely enables Lefty to antagonize Nodal signaling by preventing the obligatory interaction between EGF-CFCs and the type I–type II receptor complex (Figure 1B,C) [11,12].

Does Lefty Affect EGF-CFC-Dependent Mechanisms beyond TGFβ Signaling?

The initial descriptions of the *Xenopus* and zebrafish Leftys characterized them as Activin antagonists [5,8,9]. The EGF-CFC factor Cripto can antagonize Activin signaling by interacting with Activin and its receptor complex [15]. By binding Cripto, Lefty might alter or inhibit the interaction of Activin with Cripto and subsequently influence Activin signaling. However, Chen and Shen [11] and Cheng *et al.* [12] found that, in the presence of EGF-CFCs, Lefty cannot diminish Activin signaling, as measured by a Smad2-responsive luciferase reporter [11,12]. The previous observations that misexpression of Activin or Activin receptors suppresses Lefty gain-of-function phenotypes might be explained by the fact that Activin can activate TGFβ signaling in an EGF-CFC-independent manner and thereby circumvents the Lefty–EGF-CFC-mediated reduction of Nodal/Vg1/GDF signaling.

Cripto can also activate Akt and mitogen-activated protein (MAP) kinase pathways independently of TGFβ signaling [1,15]. Interestingly, Ulloa *et al.* [16] have shown that Lefty can activate MAP kinase as well. These observations suggest that Lefty–Cripto interactions might play a role in MAP kinase activation (Figure 1B,C). Additional studies are necessary to clarify whether Lefty–EGF-CFC complexes are capable of direct intracellular signaling.

Does Lefty Bind TGFβs other than Nodal?

Leftys were predicted to be incapable of forming homodimers or heterodimers with other TGFβ ligands,

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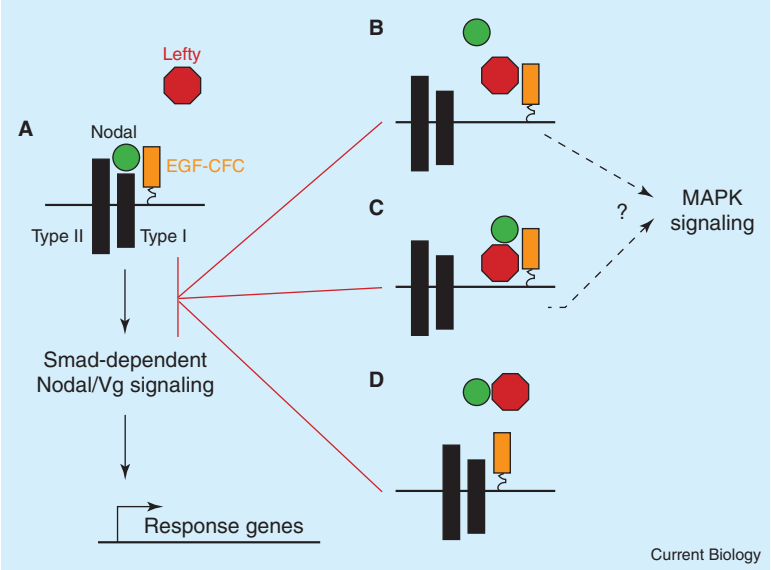

Figure 1. Direct interaction of Lefty with EGF-CFCs and Nodal antagonizes Nodal signaling.

(A) Nodal signaling is an EGF-CFC-dependent process. The binding of Nodal by EGF-CFC co-receptors facilitates the interaction of Nodal and the type I–type II Nodal receptor complex. This extracellular interaction leads to the Smad-dependent upregulation of Nodal response genes. (B–D) Lefty inhibits Nodal signaling by preventing the association of Nodal with the type I–type II receptor complex. This inhibition results from the direct interaction of Lefty with the EGF-CFC co-receptor (B,C) or with Nodal (C,D). Additionally, both Lefty and EGF-CFCs can activate MAP kinase signaling, suggesting a possible role for Lefty–EGF-CFC interactions in this process (B,C). For simplicity, Lefty and Nodal are depicted as monomers.

as they lack the canonical cysteine residue that stabilizes TGFβ dimers [2,5]. However, Chen and Shen [11] found that Lefty and Nodal can directly interact in solution, revealing a second potential mechanism of Lefty-dependent Nodal antagonism. This interaction likely antagonizes Nodal signaling by preventing Nodal from binding its receptor complex (Figure 1C,D), as evidenced by the ability of Lefty to abrogate the co-immunoprecipitation of Nodal with the type II receptor [11]. Alternatively, Lefty–Nodal interactions might affect the endoproteolytic processing of Nodal and subsequently impact Nodal activity.

Many studies have suggested that Lefty can antagonize TGFβ signaling pathways other than Nodal. The ability of Lefty to inhibit Vg1/GDF, but not Activin or TGFβ1, signaling was discussed above. Lefty has also been implicated in BMP signaling [3,7,10]. In light of the discovery of Lefty–Nodal interaction, can Lefty biochemically interact with Vg1/GDFs, BMPs or itself? If so, how will these interactions affect signaling? The identification of the domains in Nodal and the EGF-CFCs necessary for Lefty binding will allow the '*in silico*' selection of other candidates for Lefty interaction.

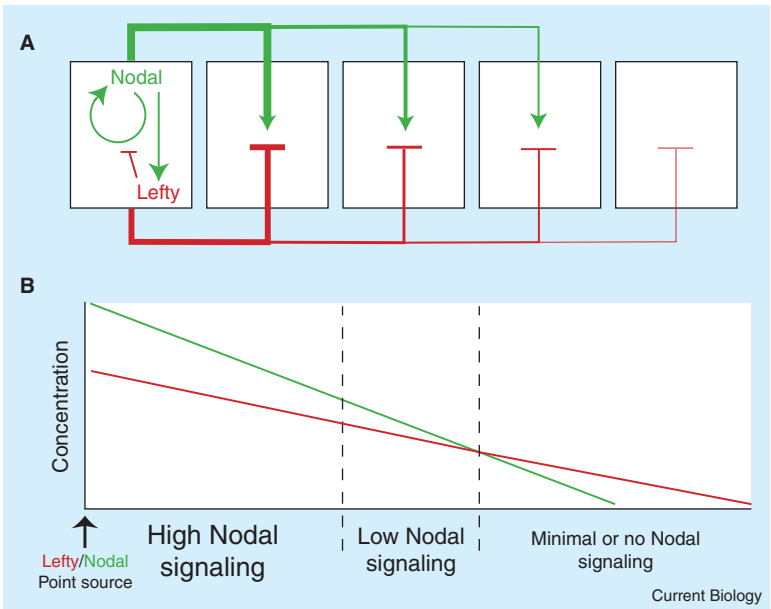

Figure 2. Lefty–Nodal reaction–diffusion establishes Nodal signaling gradients.

The Turing reaction–diffusion model hypothesizes that interactions between an activator and an inhibitor control signaling gradients within a field of cells and subsequently pattern them. Nodal–Lefty interactions exemplify several aspects of this model [1,17]. First, the activator (Nodal) activates its own production. Second, the activator (Nodal) activates its inhibitor (Lefty). Third, the inhibitor (Lefty) blocks activator (Nodal) autoactivation. Fourth, the inhibitor (Lefty) acts at a distance to restrict the effective range of the activator (Nodal). (A) Schematic illustration of Lefty–Nodal reaction–diffusion. The black squares represent a field of cells within a Nodal-signaling gradient. The left-most cell is the Nodal/Lefty point source, and the first three properties of Nodal–Lefty reaction–diffusion are depicted within. The red lines and bars emanating from this cell depict the fourth property, the long-range activity of Lefty. The green lines and arrows depict Nodal signaling. Note that Lefty is depicted to act farther from its source than Nodal [2,17]. Nearest to the point source, Nodal concentrations predominate over those of Lefty, resulting in high-level Nodal signaling. Farther from the source, the ratios of Lefty to Nodal increase and result in low-level Nodal signaling. Farthest from the source, Lefty concentrations predominate and effectively inhibit Nodal signaling. (B) Graphic depiction of the Nodal (green line) and Lefty (red line) concentration gradients in A.

Do Lefty–Nodal–EGF-CFC Interactions Shape Nodal Signaling Gradients?

The interactions between Lefty and Nodal have been proposed to resemble a Turing reaction–diffusion model, in which an activator (Nodal) and an inhibitor (Lefty) are emitted from a point source to pattern fields of cells during development (Figure 2) [1,17]. One postulate of this model is that the inhibitor acts over a long range to restrict the effective range of the activator. Accordingly, several groups [2,6,17] have shown that Lefty acts over long distances to inhibit long-range Nodal signaling. The ability of the membrane-bound EGF-CFCs to physically interact with Nodal and Lefty suggests that EGF-CFCs might directly modulate the range of these secreted ligands. The challenge will be to experimentally separate the known role of EGF-CFCs in Nodal signal transduction from potential roles in the regulation of Lefty and Nodal diffusion.

Are Lefty–EGF-CFC Interactions Involved in Cancer?

Cripto overexpression has been strongly implicated in tumorigenesis, but it is unclear how Cripto contributes to this process [15]. Additionally, Oep has recently been shown to affect cell migration, a process integral to metastasis [18]. Might Lefty–EGF-CFC interactions facilitate or inhibit oncogenesis? Alterations in Lefty expression have been detected in multiple tumor types [19,20], and Ulloa and Tabibzadeh [3] briefly noted that introduction of Lefty to tumor cells reduces tumor growth in nude mice. The ability of Leftys and EGF-CFCs to affect TGFβ and MAP kinase signaling, both of which are intimately involved in oncogenesis, strongly suggests that future studies on the role of EGF-CFCs in cancer should explore possible functions for Lefty as well.

Chen and Shen [11] and Cheng *et al.* [12] have provided two key insights into the molecular mechanisms governing the Lefty-dependent antagonism of TGFβ signaling. First, Lefty appears to exclusively antagonize EGF-CFC-dependent TGFβ signaling (Nodal/Vg1/GDF). Second, this antagonism is achieved via interaction of Lefty with EGF-CFC co-receptors (Cripto, Cryptic, Oep) or Nodal. These interactions pose a number of interesting questions, as outlined above, that need to be addressed if the 'cryptic' mechanisms of Lefty function and diffusion are to be fully 'decoded'.

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